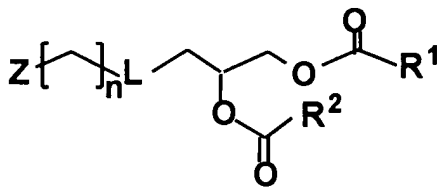
Amendments to the Specification

On page 4, please replace the paragraph starting on line 13 with the following:

Accordingly, in one aspect, the invention includes a composition for administration of a nucleic acid, comprising

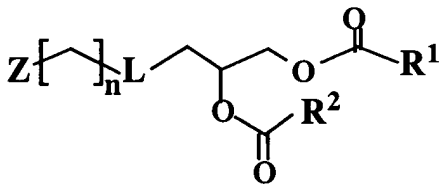
(a) liposomes comprised of (i) a lipid having the formula:



where each of R^1 and R^2 is an alkyl or alkenyl chain having between 8-24 carbon atoms, and each of R^1 and R^2 are independently selected; n = [[1]]0-20; L is selected from the group consisting of (1) -X-(C=O)-Y-[[CH_2-]], (2) -X-(C=O)-, and (3) -X-[[CH_2-]], where X and Y are independently selected from oxygen, NH and a direct bond; Z is a weakly basic moiety that has a pK of less than 7.4 and greater than about 4.0.

On page 10, please replace the paragraph starting on line 19 with the following:

The cationic-neutral lipid included in the liposomes of the present invention is generally a lipid represented by the following structure:



where each of R^1 and R^2 is an alkyl or alkenyl chain having between 8-24 carbon atoms; n = [[1]]0-20 and in a preferred embodiment is between 1-10; L is selected from the group consisting of (i) -X-(C=O)-Y-[[CH_2-]], (ii) -X-(C=O)-, and (iii) -X-[[CH_2-]], where X and Y are independently selected from oxygen, NH and a direct bond; and Z is a weakly basic moiety that has a pK of less than 7.4 and greater than about 4.0.

On page 13, please replace the paragraph starting on line 4 with the following:

Also included as embodiments of Z are certain acyclic amine compounds, such as N,N'-dimethylguanidine and various substituted hydrazines, such as trimethylhydrazine, tetramethylhydrazine, 1-methyl-1-phenylhydrazine, 1-naphthalenylhydrazine, and 2-, 3-, and 4-methylphenyl hydrazine, all of which are reported to have pKa' between 4.5 and 7.0. Alicyclic compounds having pKa's in this range include 1-pyrrolidineethanamine, 1-piperidineethanamine, hexamethylenetetramine, and 1,5-diazabicyclo[~~3.3.3~~ 3,3,3]undecane.

On page 13, please replace the paragraph starting on line 24 with the following:

The lipids of the invention include a neutral linkage L joining the Z moiety and the tail portion of the lipid. Linkage L is variable, and in preferred embodiments is selected from a carbamate, an ester, an urea amide, a carbonate, ~~a urea,~~ an amine, and an ether. In a preferred lipid prepared in support of the invention, a carbamate linkage, where L is -X-(C=O)-Y-[[CH_2-]], X being NH and Y being oxygen, was prepared.

On page 14, please replace the paragraph starting on line 4 with the following:

The lipid of the invention can be prepared using standard synthetic methods. As mentioned above, in studies performed in support of the invention, a lipid having the structure shown above, where Z is an imidazole, [[N]]n = 2, L is a carbamate and $R^1 = R^2 = C_{17}H_{35}$, was prepared. A reaction scheme for preparation of the exemplary lipid is illustrated in Fig. 1 and details of the synthesis are provided in Example 1. Briefly, the *para*-nitrophenyl carbonate of 1,2-distearoyl glycerol (Compound III) was prepared from 1,2-distearoyl-sn-glycerol (Compound I) and *para*-nitrophenyl chloroformate (Compound II) and reacted with histamine (Compound IV) to yield a lipid (Compound V) having a imidazole moiety linked to a distearoyl tail via a carbamate linkage. A similar route, using glycerol in place of 1-amino-2,3-propanediol, can be used to produce a carbonate-linked product (L = -O-(C=O)–O–CH_2–).

On page 14, please replace the paragraph starting on line 19 with the following:

Preparation of the lipid having other linkages is readily done by those of skill in the art using conventional methods. Other linkages include ether (L = -O-CH_2-)and ester linkages (L = -O-(C=O)-), as well as urea amide, ~~urea~~ and amine linkages (*i.e.*, where L = –NH–(C=O)–NH–, –NH–(C=O)–[[CH_2–]], [[-NH-(C=O)-NH-CH_2–]], or –NH–[[CH_2–]]). A keto linkage, where [[L]]X is a direct bond, is also possible. Figs. 2A-2B illustrate preparation of an ~~ether~~amine-linked lipid (Fig. 2A) and a~~n ester-linked~~ lipid having an NH- containing linkage (Fig. 2B). In Fig. 2A, the terminal amine of histamine is reacted with ~~with~~ glycidyl chloride, the resulting epoxide is hydrolyzed, and the resulting diol is acylated.

On page 14, please replace the paragraph starting on line 30 with the following:

In Fig. 2B, a~~n ester-linked~~ lipid having an NH- containing linkage ~~(L = –O-(C=O)– or –O-(C=O)-CH_2–)~~ is prepared, for example, by reacting histamine with an activated derivative of glyceric acid acetonide (2,2-dimethyl-1,3-dioxolane-4-carboxylic acid) or the four-carbon homolog, 2,2-dimethyl-1,3-dioxolane-4-acetic acid, as shown. The diol is then deprotected and acylated.

On page 15, please replace the paragraph starting on line 3 with the following:

Figs. 2C and 2D show other reaction schemes for preparation of pH-responsive lipids in accord with the invention. In Fig. 2C, 4-nitrobenzoic acid is condensed with 1-amino-2,3-propanediol, giving an amide linkage; the diol is acylated and the nitro group reduced to an amine to give the ~~the~~ product, a lipid-aniline conjugate. In Fig. 2D, the initial condensation reaction is between an alcohol (diacylgycerol) and an isocyanate, giving a carbamate linkage in the product.

On page 15, please replace the paragraph starting in line 3 with the following:

Figs. 3A-3D show various structures of pH-responsive lipids in accord with the invention, where Figs. 3A-3B show further lipids having an aromatic amine as the "Z" moiety, and Figs. 3C-3D show lipids having an aminosugar attached to a lipid.

Synthesis of these lipids can be readily performed by those of skill in the art using commercially available starting materials. For example, the product of Fig. 3A may be prepared by reaction of m-nitrobenzyl bromide and a diacylglycerol, giving the ether linkage, followed by reduction of the nitro group. The product of Fig. 3B is prepared from commercially available (2-nitrophenyl)-1,2-ethanediol by acylation of the diol and reduction of the nitro group. To prepare the aminosugar-lipid conjugate shown in Fig. 3C, D-glucose (furanose form) is protected by reaction with two molecules of acetone, and the free hydroxyl group is sequentially reacted with TsCl, sodium azide, and iodine to give an intermediate nitro compound. The exocyclic diol is deprotected and acylated, and the nitro group reduced to the amine. The compound of Fig. 3D can be prepared in a similar manner from D-galactose.

On page 18, please replace the paragraph starting on line 21 with the following:

In this reaction scheme, mPEG-methyl-dithiobenzyl- nitrophenyl chloroformate was reacted with DSPE to form the desired compound. The nitrophenyl chloroformate moiety in the mPEG-methyl-dithiobenzyl-nitrophenyl chloroformate compound acts as a leaving group to yield the desired product upon reaction with a selected lipid. The compound can also be produced by reaction with a compound such as mPEG-methyl-dithiobenzyl-R^3, where R^3 represents a leaving group joined through a linking moiety to the benzene ring. The leaving group is displaced upon reaction with an amine-containing ligand, such as DSPE, a polypeptide or an amine-containing drug. The leaving group is selected according to the reactivity of the amine in the ligand, and is preferably derived from various acidic alcohols that have a hydroxy- or oxy-containing leaving group. These include chloride, *p*-nitrophenol, *o*-nitrophenol, N-hydroxy-tetrahydrophthalimide, N-hydroxysuccinimide, N-hydroxy-glutarimide, N-hydroxynorbornene-2,3-dicarboxyimide, 1-hydroxybenzotriazole, 3-hydroxypyridine, 4- hydroxypyridine, 2-hydroxypyridine, 1-hydroxy-6-trifluoromethylbenzotriazole, imidazole, triazole, N-methyl-imidazole, pentafluorophenol, trifluorophenol and trichlorophenol.

On page 27, please replace the paragraph starting on line 21 with the following:

Examples 8-12 describe other studies performed in support of the invention, where FGF-targeted liposome/DNA complexes were administered to mice inoculated with Lewis lung tumors or to mice injected with Matrigel, an FGF-angiogenic endothelial cell model for tumor vasculature targeting. In these studies, liposomes in accord with the invention were composed of the neutral-cationic lipid HDSG (Compound V of Fig. 1) and either cholesterol or PHSPC. PEG-DTB-lipid was also included in the formulations in accord with the invention. A cationic lipid was also included in the complexes, to determine the effect of the cationic lipid on complex stability and transfection efficiency. Two cationic lipids were utilized, DOTAP and N^2-[N^2, N^5-bis(3-aminopropyl)-L-ormithyl]-N,N-dioctadecyl-L-glutamine tetrahydrotrifluoroacetate, referred to herein as "GC33".

On page 30, please replace Table 5 with the following:

Formulation No. (See Example 11 for details)	**Targeting Ligand**	**Luciferase Expression (pg luciferase/mg protein)**		
		Matrigel	**Lung**	**Liver**
Formulation No. 11-1 (DOTAP/Chol)	none	35.0	102929.3	116.9
Formulation No. 11-2 (HDSG/PHSPC)	FGF	34.3	1.6	2.1
Formulation No. 11-3 (HDSG/DOTAP/PHSPC)	FGF	45.5	1.7	2.3
Formulation No. 11-4 (HDSG/DOTAP/CHOL)	none	40.9	62.0	3.0
Formulation No. 11-5 (HDSG/DOTAP/CHOL)	FGF	21.4	61.2	4.0
Formulation No. 11-6 (HDSG/DOTAP/CHOL/FC-PEG)	FGF	64.5	4.0	1.6
Formulation No. 11-7 (HDSG/DOTAP/CHOL)	sialyl Lewix-^{X}	42.8	34.1	2.2
Formulation No. 11-8 (HDSG/DOTAP/CHOL)	FGF	38.1	12.4	2.6

On page 30, please replace Table 6 with the following:

Formulati n No. (See Example 12 for details)	Targeting Ligand	Luciferase Expressi n (pg luciferase/mg protein)[1]		
		Tumor	Lung	Liver
Formulation No. 12-1 (DOTAP/Chol)	none	72.1	273214.0	174.4
Formulation No. 12-2 (HDSG/PHSPC) (>5 days)	FGF	17.5	9.2	3.5
Formulation No. 12-3 (HDSG/PHSPC) (1 day)	FGF	32.8	8.4	3.9
Formulation No. 12-4 (HDSG/DOTAP/Chol/FC-PEG) (>5 days)	none	22.3	12.2	6.2
Formulation No. 12-5 (HDSG/DOTAP/Chol/FC-PEG) (1 day)	FGF	33.4	23.2	2.5

[1]expression was measured 24 hours after liposome ~~admininstrataion~~administration of Formulation Nos. 12-1, 12-3 and 12-5, and five days after administration of Formulation Nos. 12-2 and 12-4.

On page 30, please replace the paragraph starting on line 11 with the following:

The data in Tables 2-6 show that using the HDSG neutral-cationic lipid rather than a conventional cationic lipid achieved an extended biodistribution of the DNA-liposome complexes. This feature can be seen by comparing, for example, the luciferase expression in the lung and tumor for [[f]]Formulation [[n]]Nos. 9-1 and 9-2. This enhanced biodistribution achieved when the neutral cationic lipid was included in the liposomes even when a cationic lipid was present in addition to the neutral-cationic lipid, as seen when the results for Formulation Nos. 10-1 and 10-2 are compared.

On page 31, please replace the paragraph starting on line 8 with the following:

The effect of the FGF targeting ligand on *in vivo* gene expression can be seen by comparing Formulation Nos. 9-2 with 9-3 and Formulation Nos. 9-5 with 9-6 and Formulation Nos. 10-3 with 10-4. In these formulations, luciferase expression was higher, and sometimes significantly higher, when the complexes included the FGF targeting ligand.

On page 31, please replace the paragraph starting on line 14 with the following:

A comparison of Formulation Nos. 9-7, 9-8, and 9-9 with Formulation Nos. 10-5, 10-6, and 10-7 show that reducing the amount of GC33 from 22.5 mole percent to

11.25 mole percent shifts the luciferase expression from the lung to the tumor or Matrigel. This shift of expression level is due to a reduction in surface charge of the liposome-DNA complexes, indicating that the absence of surface charge at physiological pH increases blood retention time and changes the tissue distribution of the complexes after systemic administration.

On page 31, please replace the paragraph starting on line 23 with the following:

Examples 13-15 describe *in vivo* administration of various liposome formulations to mice bearing a KB tumor. In these studies, the complexes included a folate ligand for targeting to the folate-expressing tumor cells. The formulations are described in Example 13-15 and the ~~luciferease~~luciferase expression in the tumor lung and liver following intravenous administration are shown in Table 7-9.

On page 44, please replace the paragraph starting on line 10 with the following:

3. 1-(mercaptomethyl)ethylammonium chloride. 5-Methylthiazolidine-2-thione (6.5 g, 49 mmol) was placed in a 250 ml round-bottom-flask. A solution of aqueous hydrochloric acid (40 ml, 18% in H_2O) was added and the flask was heated in an oil bath. The reaction refluxed (120°C) for one week. Three times throughout the week 1 ml of concentrated hydrochloric acid was added. The reaction was monitored using TLC with ethyl acetate as eluent. They were visualized using UV, ninhydrin, and iodine vapors. Through most of the week the reaction was a heterogeneous mixture, with the starting material as oil which was denser than water. After one week the oil starting material was gone, although still visible on TLC. The reaction was removed from heat and allowed to cool to room temperature, and then was refrigerated to crystallize starting material. The crystallized starting material was filtered. Filtrate was evaporated and it was dried over P_2O_5 and NaOH to remove all water and [[HCl]]HCl. The crude product was washed with two portions of diethyl ether (50 ml each) to remove all starting material. It was again dried over P_2O_5. Yield: 2.83 g (45%). ^{1}H NMR (D_6-DMSO): δ

1.33 (d, C*H*$_3$, 3H); δ 2.92 (m, N-C*H*$_2$, 2H); δ 3.12 (m, S*H*, 1H); δ 3.18 (m, R$_3$-C*H*, 1H); δ 8.23 (bs, N*H*$_3$, 3H). Melting point: 80-82°C (lit: 92-94).

On page 47, please replace the paragraph starting on line 12 with the following:

Ethylene diamine (42 µl, 0.63 mmol, 5 fold excess), and pyridine (200 µl, were added in $CHC1_3$ (1 ml). 2-disteroyl-*sn*-p-nitrophenyl carbonate (100 mg, 0.13 mmol) was dissolved in $CHCl_3$ (1 ml) and added dropwise to ethylene diamine solution with a ~~pastuer~~pasteur pipette at 0°C (ice water) and continued overnight (16 h). TLC ($CHC1_3$: MeOH: H_2O 90: 18: 2, and $CHC1_3$: MeOH = 90: 10) showed that the reaction was complete. Solvent was evaporated to remove pyridine. Then the product mixture was dissolved in $CHC1_3$, loaded onto the column (Aldrich, Silica gel, 60°A, 200-400 mesh), and eluted with $CHC1_3$: CH_3COCH_3, and $CHC1_3$: MeOH gradient, $CHC1_3$: CH_3COCH_3 = 90: 10, 60 ml (upper spot eluted); $CHC1_3$: NeOH = 90: 10, 60 ml (product eluted). Fractions containing pure product were combined and evaporated. Tert-butanol was added and dried in *vacuo* over P_2O_5. Yield: 64 mg (75%). ^{1}H NMR (DMSO-d_6, 360 MHz) δ .83 (t, end CH_3, 6H); 1.22 (s, 28xCH_2, 56H); 1.51 (m, C*H*$_2$CH$_2$CO, 4H); 2.25 (2xt, C*H*$_2$CO, 4H); 2.83 (m, H$_2$NC*H*$_2$CH$_2$NH, 2H); 3.21 (m, H$_2$NCH$_2$C*H*$_2$NH, 2H); 4.10-4.14 (m & cis d, COOC*H*$_2$CHC*H*$_2$, 4H); 5.17 (m, OCOOC*H*$_2$CH, 1H); 7.78 (m, *H*$_2$NCH$_2$CH$_2$NH, 2H).

On page 49, please replace the paragraph starting on line 4 with the following:

FGF or folate ligands were conjugated to maleimide-PEG-DSPE, according to procedures known in the art (Gabizon, A. *et al*, *Bioconjugate Chem.*, 10:289 (1999)). DNA-liposome complexes were incubated with micellar solutions of mPEG-DSPE, FGF-PEG-DSPE or folate-PEG-DSPE with continuous stirring for 20 ~~minites~~minutes to achieve insertion of the ligand-PEG-lipid into the pre-formed liposomes.

On page 49, please replace the paragraph starting on line 15 with the following:

KB tumor cells (1 million cells) were inoculated subcutaneously to the flank of nude mice. The mice were fed a reduced folate diet to upregulate the ~~exression~~expression of folate receptors on the KB tumor cells. This model was used for folate-conjugated liposome-DNA complexes to target tumor vasculature angiogenic endothelial cells.

On page 51, please replace line 3 with the following (additions in bold):

C. In vivo Administration

On page 51, please replace line 26 with the following (additions in bold):

B. Liposome Formulations

On page 55, please replace line 7 with the following (additions in bold):

B. Liposome Formulations

On page 58, please replace line 12 with the following (additions in bold):

B. Liposome Formulations

On page 61, please replace line 8 with the following (additions in bold):

B. Liposome Formulations

On page 63, please replace the paragraph starting on line 6 with the following:

Six-days after [[i]]implantation of Matrigel, 24 mice were randomized into treatment groups (n=3) for treatment with one of the formulations, Formulation No. (11-1) through Formulation No. (11-8). The liposome-DNA complexes were administered intravenously at a dose of 200 μg DNA plasmid. Twenty-four hours after administration of the FGF-targeted liposome-DNA complexes, luciferase expression in the matrigel, lung and liver was measured. The results are shown in Table 5.

On page 64, please replace line 1 with the following (additions in bold):

B. Liposome Formulations

On page 65, please replace the paragraph starting on line 4 with the following:

Nine-days after inoculation with tumor cells, 15 tumor-bearing mice were randomized into treatment groups (n=3) for treatment with one of nine formulations, Formulation No. (12-1) through Formulation No. (12-5). The liposome-DNA complexes were administered intravenously at a dose of 100 μg DNA plasmid. Twenty-four hours after administration of Formulation Nos. 12-1, 12-3 and 12-5, and five days after administration of Formulation Nos. 12-2 and 12-4, luciferase expression in the tumor, lung and liver was measured. The results are shown in Table 6, where expression was measured 24 hours after liposome ~~admininstrataion~~administration of Formulation Nos. 12-1, 12-3 and 12-5, and five days after administration of Formulation Nos. 12-2 and 12-4.

On page 65, please replace line 23 with the following (additions in bold):

B. Liposome Formulations

On page 68, please replace line 18 with the following (additions in bold):

B. Liposome Formulations

On page 70, please replace line 18 with the following (additions in bold):

B. Liposome Formulations